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Date of Signature and Deposit: 4/14/05

Jean C. Baker, Reg. No. 35,433

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Diana Downs
Jeff A. Gralnick

Serial No.: 09/955,502 Group Art Unit: 1645

Filed: 09/18/2001 Examiner: Patricia Ann Duffy

Title: METHOD FOR PREVENTING SUPEROXIDE DAMAGE TO CELLS AND OXYGEN-LABILE PROTEINS File: 960296.97559

DECLARATION UNDER 37 CFR §1.132

Commissioner for Patents
P O Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Diana M. Downs, on oath say and declare that:

1. I am the same Diana M. Downs who is one of the named inventors of the above-identified patent application. I make this declaration in support of that patent application. I am currently employed by the University of Wisconsin-Madison where I am a professor in the Department of Bacteriology. I have worked as a research scientist specializing in the general area of bacterial metabolism and physiology for 18 years. I have published extensively in this area. A copy of my professional biography is attached as Exhibit A.

2. I have reviewed the Office Action issued by the U.S. Patent and Trademark Office (USPTO) on October 18, 2004 for this application. I understand that currently Claims 1, and 7-10 are rejected for allegedly failing to comply with the USPTO's written description requirement. Specifically, in this regard, I wish to disagree with the Examiner's assertion that:

"...the written description is limited to methods that reduce superoxide damage to

a eubacterial cell comprising vector-based over-expression of the endogenous YggX gene from said cell, wherein said overexpression renders the cells more resistant to superoxide damage."

From this assertion it appears that the Examiner was not convinced that the methods for reducing superoxide damage to a eubacterial cell would be equally applicable for vector-based over-expression to all YggX genes (i.e., exogenous and endogenous). Accordingly, this Declaration is submitted to provide evidence that vector-based over-expression of an exogenous (referred to herein as "heterologous") and endogenous YggX gene does indeed render cells more resistant to superoxide damage.

3. In general, I note that use of heterologous expression systems in *E. coli* has become prevalent among researchers in microbiology and other molecular fields, particularly for identifying genetic or biochemical functions from less tractable systems. Furthermore, the sophistication of technologies currently available in the field, in combination with comparative genomic capabilities have almost made *E. coli*-based expression systems a required lab reagent. It has become relatively routine for investigators interested in "non-model" systems to use *E. coli* for expressing, purifying and analyzing mutant and wild-type proteins. The literature is full of examples where investigators have expressed proteins of interest in *E. coli.*

4. To further demonstrate that heterologous vector-based over-expression is widely accepted and practiced in the art of bacteriology, I have cited the following publications, the first of which is a paper that I have authored: (1) Downs, D.M. (2003) Genomics and Bacterial Metabolism. Current Issues in Molecular Biology 5:17-25; (2) Weinstock, G. M., et al. (2000) From Microbial Genome Sequence To Applications. Res Microbiol 151(2):151-158; (3) Handelsman, J., et al., (1998) Molecular Biological Access To The Chemistry Of Unknown Soil Microbes: A New Frontier For Natural Products. Chem Biol 5(10):R245-249; (4) Rondon, M. R., et al., (1999) Toward Functional Genomics In Bacteria: Analysis Of Gene Expression In *Escherichia Coli* From A Bacterial Artificial Chromosome Library Of *Bacillus Cereus*, PNAS USA 96(11):6451-6455; (5) Park, J. H., et al., (2004) Characterization of Two Kinases Involved in Thiamine Pyrophosphate and Pyridoxal Phosphate Biosynthesis in *Bacillus subtilis*: 4-Amino-5-Hydroxymethyl-2-Methylpyrimidine Kinase and Pyridoxal Kinase, J. of Bacteriology 186(5):1571-1573; (6) Woodson, J.D., et al., (2003) A New Pathway for Salvaging the Coenzyme B_{12} Precursor

Cobinamide in Archaea Requires Cobinamide-Phosphate Synthase (CbiB) Enzyme Activity, J. of Bacteriology 185(24):7193-7201; (7) Grimek, T.L., et al., (2004) The *acnD* Genes of *Shewenella oneidensis* and *Vibrio cholerae* Encode a New Fe/S-Dependent 2-Methylcitrate Dehydratase Enzyme That Requires *prpF* Function In Vivo, J. of Bacteriology 186(2):454-462; and (8) Woodson, J.D., et al., (2004) CbiZ, an amidohydrolase enzyme required for salvaging the coenzyme B_{12} precursor cobinamide in archaea, PNAS USA 101(10):3591-3596. Each of these publications describe work where proteins have been expressed (and characterized) in heterologous systems, helping to demonstrate that the invention should not be limited to methods that reduce superoxide damage to a eubacterial cell having vector-based over-expression of only the endogenous YggX gene.

5. Furthermore, these publications illustrate the facility and value of heterologous expression to define function by *in vivo* complementation. Specifically, at pg. 7198 of Woodson et. al., (2003), the authors identify the ability of archaeal genes *cbiP* and *cbiB* to complement mutants of *Salmonella enterica* with a known defect. Similarly, in Grimek and Escalante-Semerena (2004), the authors demonstrated the function of two genes from *Vibrio cholerae* and *Shewenella onidenes* by the same general method (see pg. 454). Also, at pg. 3593 of Woodson and Escalante-Semerena (2004), function of an additional archaeal gene was determined by complementation of defined Salmonella mutants. Another paper which specifically illustrates the value (and standard nature) of using tractable systems (i.e., *E. coli*) to facilitate protein production for characterization and functional analysis is Park et. al. (2004). Park et. al. describes the expression of proteins (ThiD and YjbV) from *Bacillus subtilis* in *E. coli* cells for purposes of purification and functional analysis. The ability of these authors (and many others) to conclude function of a gene product from results in a heterologous system emphasizes the similarity of basic biological processes in all organisms supports the assumption that the YggX gene or homologs thereof will function similarly in multiple heterologous backgrounds.

6. Although, there are several practical reasons for the wide-spread use of heterologous expression systems in *E. coli,* I believe the most important reason is that *E. coli* allows the efficient overexpression of a variety of different proteins by permitting metabolic machinery between organisms to be shared. I believe that this sharing of metabolic machinery between organisms is what allows a protein that is expressed in a heterologous system to behave as if expressed it is expressed in an endogenous system. Exceptions include

proteins which require novel cofactors or specialized modifications that may be present only in the native host, such as proteins requiring the assembly of a specialized metabolic cofactor. It is widely understood in the art that such specialized host requirements are the exception and not the rule. I note that YggX has no associated cofactor that is required for function – the polypeptide is sufficient.

7. More specifically, with respect to the heterologous vector-based over-expression of YggX gene, the application itself provides a specific example of "mixing and matching" between *Salmonella entêrica* and *E. coli (*see, paragraphs [0029] to [0036]). Accordingly, the specification itself demonstrates that the invention is not simply limited to methods that reduce superoxide damage to a eubacterial cell using vector-based over-expression of an endogenous YggX gene from the cell. Indeed, as we have noted and recited in the specification (see paragraph [0025]), while we have performed experiments thus far in bacterial cells we anticipate a similar mechanism of protection to occur with YggX in other cell types, including yeast, mammalian and plant cells. This expectation is due to the similarity of structure, function and oxygen lability of [Fe--S]-containing proteins in each of these cell types.

8. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Further declarant sayeth not.

Dated: 3/15, 2005

Diana M. Downs

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Diana M. Downs	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Utah, SLC, UT	B.S.	1981	Biology
University of Utah, SLC, UT	Ph.D.	1987	Bacterial Genetics(Biology)
University of Utah, SLC, UT	Postdoc	1987-88	Bacterial Genetics
Univ. of Wisconsin-Madison	Postdoc	1988-91	Biochemistry

Professional Experience

7/01-present	Professor, Department of Bacteriology, University of Wisconsin, Madison
7/98-6/01	Associate Professor, Department of Bacteriology, University of Wisconsin, Madison
7/93-6/98	Assistant Professor, Department of Bacteriology, University of Wisconsin, Madison

Awards

NIH Postdoctoral Fellowship, 10/89-5/92.
Shaw Scientist Award, 1993.
WALSAA Outstanding Advisor Award, 1998
Hilldale Undergraduate/Faculty Research Award, 1998, 2001, 2003
Dupont Educational Aid Grant 1998-99
Finalist, McDonnell Centennial Fellowship 1998
College of Agriculture and Life Sciences, Pound Research Award, 1999
21st Century Scientist Award for Complex System Analysis, 2001
Jung Excellence in Teaching Award (CALS) 2002
SC Johnson Distinguished Fellowship 2002-2005

Publications (2001-present)

1. Zilles, J., T. J. Kappock, J. Stubbe, and D. M. Downs. 2001. Altered routing in a novel class of mutants defective in aminoimidazole ribonucleotide synthetase in *Salmonella enteria* serovar typhimurium. J. Bacteriol. **183**:2234-2240.
2. Gralnick, J. A., and D. M. Downs. 2001. Protection from superoxide damage associated with an increased level of a particular bacterial protein. P.N.A.S. **98**:8030-8035
3. Downs, D. M. 2001."Biochemical Genetics" in Encyclopedia of Genetics. Ed. S. Brenner, J. Miller. Academic Press. London (INVITED)
4. Rubio, A. and D. M. Downs. 2002. Elevated levels of ketopantoate hydroxymentyltransferase (PanB) lead to a physiologically significant increase of CoA levels in *Salmonella enterica* Serovar Typhimurium. J. Bacteriol. **184**:2827-2832.
5. Allen, S., J. L. Zilles and D. M. Downs. 2002. Metabolic flux in both the purine mononucleotide and histidine biosynthetic pathways can influence synthesis of the hydroxymethyl pyrimidine moiety of thiamin in *Salmonella enterica*. J. Bacteriol. **184**:6130-6137.

6. Skovran, E. and D. M. Downs. 2003. Lack of ApbC or ApbE proteins results in a defect in Fe-S cluster metabolism in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **185:**98-106.
7. Dougherty, M. and D. M. Downs. 2003. The *stm4066* gene product of *Salmonella enterica* serovar Typhimurium has aminoimidazole riboside (AIRs) kinase activity, and allows AIRs to satisfy the thiamin requirement of *pur* mutant strains. J. Bacteriol.**185:**714-720.
8. Gralnick, J.A. and D. M. Downs. 2003. The YggX protein of *Salmonella enterica* is involved in Fe(II) trafficking and minimizes the DNA damage caused by hydroxyl radicals. J. Biol. Chem. **278**:20708-20715.
9. Ramos-Solis, A.I.and D. M. Downs. 2003. Anthranilate synthase can generate sufficient phosphoribosyl amine for thiamine synthesis in vivo in *Salmonella enterica*. J. Bacteriol. **185:**5125-5132.
10. Downs, D. M. 2003. Genomics and Bacterial Metabolism. Current Issues of Molecular Biology **5**:17-25 (INVITED)
11. Schmitz, G. and D. M. Downs. 2004. Lesions in *yjgF* result in a decreased specific activity of Transaminase B (IlvE) in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **186**:803-810.
12. Beck, B., M. Huelsmeyer, S. Paul, and D.M. Downs. 2003. A mutation in the essential gene *gmk* (encoding guanlyate kinase) generates a requirement for adenine at low temperature in *Salmonella enterica.* J. Bacteriol. 6732-6735.
13. Dougherty, M. and D. M. Downs. 2004. A mutant allele of *rpoD* results in increased conversion of aminoimidazole ribotide to hydroxymethyl pyrimidine in *Salmonella enterica.* J. Bact. **186**: 4134-4037.
14. Lauhon, C. T., E. Skovran, H. D. Urbina, D. M. Downs, and L. E. Vickery. 2004. Mutations in an active site loop of *Escherichia coli* IscS result in specific defects in Fe-S cluster and thionucleoside biosynthesis in vivo. J. Biol. Chem. **279**:19551-19558.
15. Downs, D. M., Schmitz, G., E. Skovran. 200X. Probing the complex system of metabolic integration complex system. Progress in Nucleic Acid and Molecular Biology (INVITED)
16. Zhang, Y., Dougherty, M., Downs, D.M. and S.E. Ealick. 2004. Crystal Structure of an Aminoimidazole Riboside Kinase from Salmonella enterica; Implications for the Evolution of the Ribokinase Superfamily. Structure. **12**:1809-1821
17. Martinez-Gomez, N.C., Robers, M. and D.M. Downs. 2004. Mutational analysis of ThiH, a member of the radical S-adenosylmethionine (AdoMet) protein superfamily. J. Biol. Chem. **279**: 40505-40510.
18. E. Skovran, C. T. Lauhon, and D. M. Downs. 2004. Lack of YggX results in chronic oxidative stress and uncovers subtle defects in Fe-S cluster metabolism in *Salmonella enterica*. J. Bacteriol. **186:** 7626-7634.
19. Schmitz, G. and D. M. Downs. 2004. Reduced transaminase B (IlvE) activity caused by the lack of *yjgF* is dependent on the status of threonine deaminase (IlvA) in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **186**:803-810.

COPY from previous response

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Date of Signature And Deposit: 4/15/04

Attorney of Record

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Diana M. Downs, et al.
Serial No.:	09/955,502
Filed:	September 18, 2001
For:	METHOD FOR PREVENTING SUPEROXIDE DAMAGE TO CELLS AND OXYGEN-LABILE PROTEINS
Group Art Unit:	1645
Examiner:	P. Duffy

Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DIANA M. DOWNS

Dear Sir:

1. I, Diana M. Downs am one of the named inventors in the above-identified application.

2. I am also one of the authors of Gralnik, et al. (Abstracts of the General Meaning of the American Society for Microbiology, May 21 – 25, 2000) as cited by the Examiner. My co-inventor Jeffrey Gralnick is the other author.

3. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false

statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully submitted

4/5/04
Date

Diana M. Downs
Diana M. Downs